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Abstract:

Experimental forest plantations to study biodiversity–ecosystem functioning (BEF) relationships have recently been established in different regions of the world, but subtropical biomes have not been covered so far. Here we report about the initial survivorship of 26 tree species in the first such experiment in subtropical China. In the context of the joint Sino-German-Swiss Research Unit "BEF-China", 271 experimental forest plots were established using 24 naturally occurring tree species and two native commercial conifers. Based on survival inventories carried out in November 2009 and June 2010, the overall survival rate was 87% after the first 14 months. Generalized mixed-effects models showed that survival rates of seedlings were significantly affected by species richness, the species' leaf habit (deciduous or evergreen), species identity, planting date and altitude. In the first survey, seedling establishment success decreased with increasing richness levels, a tendency that disappeared in the second survey after replanting. Deciduous species had a significantly higher survival than evergreen species (93% vs. 84% in the second survey). These results have implications for establishing Though evergreen species performed less well than deciduous species with establishment rates of 84% vs. 93% in the second survey, their planting success exceeded the general expectation for subtropical broad-leave evergreen species. These results have important implications for establishing mixed species plantations for diversity conservation and improvement of ecosystem functioning in the Chinese subtropics and elsewhere. Additional costs associated with mixed -species as compared to conventional plantations also demonstrates the potential of upscaling BEF experiments to large-scale afforestation projects.

Keywords: BEF-China, biodiversity and ecosystem functioning, tree diversity experiment, Jiangxi, forest plantation success, seedling performance

1. Introduction

Planet earth has been altered by human activities in many different ways. One significant modification has been the use and overexploitation of natural forests. With a dramatic decline in natural forest, tree plantations have served as an alternative to meet the growing demands for timber and more recently growing need for ecosystem services (Bauhus et al. 2010; Puettmann and Ammer 2007). As monocultures are easily planted and are assumed to allow maximizing biomass production and minimizing cost, they have become a dominant global practice of modern forestry (Piotto 2008; Nichols et al. 2006). Monocultures are widely spread over Europe (Götmark et al. 2005), Asia (Yang et al. 2010), tropical America (Menalled et al. 1998), central America (Zeugin et al. 2010) and Australia (Forrester et al. 2005). However, monoculture plantations have been increasingly criticized with regard to the species used (including the introduction of exotic species), susceptibility to pathogens, herbivores or adverse environmental conditions and negative long-term impacts on soil fertility (Liu et al. 1998). Furthermore, new experiments on relationships between plant diversity and ecosystem functioning increasingly cast doubts on the assumption that monocultures are the way to go if maximization of biomass production is the management goal (Hooper et al. 2005; Balvanera et al. 2006). As a consequence, potential advantages of planting diverse tree mixtures have been discussed over the last decade (Piotto 2008; Scherer-Lorenzen et al. 2007b; Bauhus and Schmerbeck 2010). Assessing the impact of biodiversity on ecosystem functioning has increasingly raised interest in ecology (Biodiversity–Ecosystem Functioning or short BEF research, (Healy et al. 2008; Nadrowski et al. 2010; Hooper et al. 2005). Many studies have demonstrated positive biodiversity effects on ecosystem functioning in grasslands and other fast growing model systems (Balvanera et al. 2006; Cardinale et al. 2011). More recently, similar effects were also observed in forest ecosystems, such as increased productivity (Vilà et al. 2007), maintenance of diversity (Piotto 2008), improved water use efficiency (Forrester et al. 2010), enhanced litter decomposition (Wang et al. 2008), increased nutrient retention and cycling (Zeugin et al. 2010) and reduced risks such as insect pests (Jäkel and Roth 2004).

Only a handful of experiments with manipulated tree diversity have been established worldwide so far (Scherer-Lorenzen et al. 2005b). Similarly, among commercial plantations only a small proportion (<0.1%) are polycultures (Nichols et al. 2006). There are several reasons why mixed plantations, and in particular ones with more than two species have not gained much popularity: 1) the impracticality to produce seedlings of a multitude of different species in commercial nurseries; 2) simultaneous planting of different species with different requirements for establishment success (Scherer-Lorenzen et al. 2007b; Don et al. 2007); and 3) increasing management complexity of the established stands, involving maintenance of sub-dominant species. Without having exact figures on the additional costs involved, the common assumption is that these costs are far too high, especially for forests planted for timber production (Nichols et al. 2006). However, with the increasing recognition of the potential value of other ecosystem services provided by mixed forests such cost considerations may need to be completely revised.

Still many practical questions remain. These are particularly pressing in countries without a well-developed forestry. Which species can be used in diversity plantations? What are the main factors affecting establishment? What is the establishment success that can be expected? Globally, there are currently nine BEF projects that might provide answers to these questions (see www.treedivnet.ugent.be), representing tropical (Healy et al. 2008; Scherer-Lorenzen et al. 2007a), temperate (Scherer-Lorenzen et al. 2007b) and boreal biomes (Vehviläinen and Koricheva 2006). Among these projects, the one on biodiversity and ecosystem functioning in China (BEF-China) is the only one focusing on the species-rich subtropical area and the one with the largest species pool. The overall aim of the BEF-China project is to relate functions and services of a forest ecosystem to the biodiversity of planted tree and shrub species. In addition, diversity at other trophic levels (soil biota, herbivores, predators, pathogens) is studied as a function of manipulated tree and shrub species richness. Finally, and in particular in the initial stage of the project, the impact of abiotic variables (i.e. edaphic, climatic and topographic characteristics, here called the "ecoscape") on ecosystem functioning is contrasted with that of biotic variables (i.e. tree and shrub species richness and composition). The guiding question of the present study was: to which degree do biodiversity and abiotic variables affect the initial survivorship of 26 tree species used in the BEF-China project?

Achieving high survival rates of planted seedlings or saplings is the basic concern in many applied forestry projects. Survival rates strongly affect the project's overall costs, and hence, will finally determine the wider application of the procedure employed and its acceptance in commercial forestry. Surprisingly, while previous research on the establishment of mixed-species forest stands has mostly focused on indicators such as growth, nutrition, structure (Rouhi-Moghaddam et al. 2008; Menalled et al. 1998), little information has been provided on survival rates (but see Bosu et al. 2006; Simpson and Osborne 2006; Scherer-Lorenzen et al. 2007b; Don et al. 2007). The question which factors affect initial survival is particularly important for trees that are not commonly used in plantation forestry such as many broad-leaved tree species in subtropical China. Compared to widely-used species for plantation forestry, most of which have undergone intensive breeding and been selected for high survival rates (Vilà et al. 2005), experiments with many different species are confronted with uncertainties and a lack of knowledge on optimum planting techniques.

There are reports that planting of broad-leaved evergreen tree species suffers from high initial mortality (Tsakaldimi et al. 2007; Villar-Salvador et al. 2004; Vilagrosa et al. 2003). Similar reports can be found for some broad-leaved deciduous species (Goodman et al. 2009). Biotic interactions such as competition, complementarity and facilitation as well as herbivory, pathogen load and mycorrhiza (Healy et al. 2008), abiotic site heterogeneity involving edaphic, topographic and hydrological variation (Messaoud and Houle 2006; Montagnini 2000; Forrester et al. 2005), planting shock (Burdett 1990) and planting season (Simpson and Osborne 2006; Bosu et al. 2006; Goodman et al. 2009; Radoglou and Raftoyannis 2002) are considered as potential factors affecting survival of tree seedlings at early stages of growth. Furthermore, in the few other BEF experiments carried out with trees, survival rates were found to vary strongly among species but to be little affected by the number of species planted in a plot (Potvin and Gotelli 2008; Healy et al. 2008; Liang et al. 2007).

Making use of the first two censuses 7 and 14 months after planting of half of all plots in the BEF-China project, we asked whether initial seedling survival differed a) between different levels of species richness, in particular between monoculture and mixed species plots, b) between evergreen and deciduous species, and c) between different aspect, inclination, topographic curvature and altitude. To our knowledge, this is the first quantitative report about establishment success and other practical issues in early phases of BEF experiments with woody species worldwide and one of the few studies on forest plantations including native broad-leaved evergreen tree species from subtropical China.

2. Materials and Methods

2.1. Study site

The BEF-China experiment was established near Xingangshan Township, Dexin City of Jiangxi Province (29.08–29.11 N, 117.90–117.93 E). The climate of this region is typical of the subtropics, with mean annual temperature of 16.7 ℃ and mean annual precipitation of 1821 mm (data refer to Wuyuan County, the nearest city close to the field site, mean from 1971–2000, http://cdc.cma.gov.cn/). January is the coldest month with a mean temperature of 0.4 ℃ and July the hottest with a mean temperature of 34.2℃. The natural vegetation is characterized by subtropical forest with a mixture of evergreen and deciduous species (Bruelheide et al. 2011). However, most forested areas in this region have undergone a dramatic conversion from mixed natural forests to commercial plantations of *Pinus massoniana* and *Cunninghamia lanceolata* (Wang et al. 2007).

The BEF-China project includes two sites, A and B, at Xingangshan, planted in 2009 and 2010, respectively. In this paper, only the results from site A are reported. Site A encompasses a hilly area of 26.7 ha ranging in altitude from 105 to 275 m and in slope from 0–45 degrees. The land belongs to the Xingangshan Forest Company and prior to the experiment was covered with plantations of *Pinus massoniana* and *Cunninghamia lanceolata,* harvested at about 20-year intervals.

2.2. Experimental design

In total, it holds 271 plots that were planted with seven different levels of tree species richness. The basic plot size in horizontal projection is 666.7 m^2 (25.8 m × 25.8 m corresponding to the traditional Chinese area unit of 1 mu = 1/15 ha). There are 15, 98, 68, 40, 26, 19 and 5 plots for the richness levels of 0, 1, 2, 4, 8, 16 and 24 tree species, respectively. One set of plots is arranged in quadratic parcels of four plots to accommodate different levels of shrub species richness later on (planted after the second census in 2010). These so-called 4-mu plots have richness levels 0, 1, 2, 4, 8, 16 and 24 tree species and in sum include 12, 64, 32, 8, 4 and 4 1 mu-plots, respectively. In every 1 mu-plot, 400 individual tree seedlings were planted at equal planting distance of 1.29 m (horizontal projection). The assignment of 1-mu plots and of 4-mu plots to treatments was completely randomized (Fig. 1), as were the positions of individual tree seedlings within plots.

The basic scheme of assigning species to richness levels followed what we call a broken-stick or random-partitions design, thus making sure that every species is represented equally often at each level of species richness. This was achieved by randomly partitioning three sets of 16 species into the desired mixtures. The random-partitions design ensures that each species is selected exactly once at each level of diversity. Such a design has also been applied in other BEF experiments (Hodgson et al. 2002; Bell et al. 2005; Salles et al. 2009). In BEF-China, partitioning of lower levels of diversity was done in such a way that the less diverse communities were nested within more diverse ones, thus resulting in random extinction series. In total, the random partitions design comprised 198 plots out of the 256 plots planted with trees. An additional set of 48 plots were planted with non-random species mixtures simulating directed extinction series again passing through richness levels 16, 8, 4 and 2 in a nested way. The species sets for both the random and non-random series were drawn from the total pool of 24 native tree species of the region (Table 1). In addition, each five plots with monocultures of the commercially most important species *Pinus massoniana* and *Cunninghamia lanceolata* were included. The majority of species is characteristic of early successional stages (16 species), while four and three species mainly occur in intermediate and late stages, respectively, and further three species show no preference for any particular stage (Table 1). Species names follow the nomenclature of the Flora of China.

2.3. Seed harvest and nursery practices

As there were no commercial seedlings available for the native broad-leaved tree species used in this experiment, the project had to start with its own seed collection and nursery establishment. A wide range of indigenous species that are characteristic of the subtropical forest flora were collected. In order to ensure that a sufficient number of species and seedlings was in stock at the time of planting in 2009, seeds were continuously harvested in summer and autumn in 2007, 2008 and 2009. Until the end of 2009, a total number of 98 species had been harvested, among them the 24 broad-leave tree species used for planting (Table 1). Together with the two commercially-used conifers, a total of 26 species, 15 of them deciduous and 11 evergreens, comprising 100,400 individual tree seedlings, were planted manually. All the selected species naturally occur in the study area.

After collection, the seeds were stratified and stored in sand in a cold environment. Before sowing, they were sterilized by soaking in antimicrobial and insecticide solution. Seedlings were raised at two local nurseries. In the first year, deciduous species, known for their easy germination, were sown directly into the soil of prepared nursery beds. In contrast, evergreen species were sown into small containers filled with a rooting substrate composed of top soil from forest floor, grain chaff and fertilizer. From the second year onwards, all the seedlings were cultivated in containers, to facilitate their transfer from nursery to planting sites. Watering and weeding in the nurseries were carried out on a regular basis. To avoid excessive transpiration in summer and frost damage in winter, the seedlings in the nursery were protected with shading cloths.

2.4. Site and planting preparation

The 271 plots were arranged in a systematic grid (Fig. 1). The positions of each plot were marked by four poles, defined by using a differential GPS (Leica GPS 1200 Base-Rover-System). After clear-cutting of the previous conifer plantation, the aboveground plant biomass was removed from the experimental site. Four temporal seedling camps with shading facilities were established at locations with access to water. Because air temperature during planting was sometimes high and on some days exceeded 30 ℃, measures were taken to reduce transpiration of seedlings. To facilitate planting of bare root seedlings and to reduce their transpiration, roots and shoots were pruned based on the advice of local foresters; subsequently the roots were dipped in a soil/water suspension to which KH_2PO_4 had been added to stimulate root growth. The exact date when a plot was planted was recorded.

2.5. Planting procedure

The first planting campaign was carried out from 22 March to26 April 2009. Weather conditions in that period changed rapidly and temperatures increased within a few days from 11.7 °C on 23 March to 26.6 °C on 26 March and reached a maximum during this period of 30.6 °C on 15 April 2009 (own measurements at noon, Fig. 2). Planting sheets with randomized positions were prepared and used as guiding maps in the field to assign the individuals of each species to the right planting position. Planting was carried out plot-wise, arranging seedlings of all species in a plot according to the planting schemes. Seedlings were planted in holes of 50 x 50 cm size and >20 cm depth, the latter depending on the root length of individual seedlings. To replace dead seedlings, replanting was carried out in November 2009 (for deciduous species) and March 2010 (for the frost-sensitive evergreen species).

2.6. Weeding

Twice a year during the growing season (May–October), all undesired herbs, shrubs and tree competitors as well as coppice sprouts of the previous *Cunninghamia lanceolata* trees were removed.

Particularly noxious weeds such as *Miscanthus floridulus, Miscanthus sinensis* and bamboo (*Phyllostachys heteroclada*) were dug out with their root system. Attention was paid to seedlings of small size to prevent them from being taken out unconsciously. The cut biomass was put around the seedling as mulch.

2.7. Survival survey

The first survey of survival rate was conducted as a full census before the replanting of deciduous species in November 2009. Bamboo sticks with abbreviated species identification as well as numeric codes were installed at all positions of dead seedlings to assist later replanting. In total, 30,794 individual trees of 26 species from 224 plots out of the 256 plots planted with trees were examined during the first survey. The second survey was carried out in June 2010 after replanting had occurred. A systematic sampling scheme was applied, where 50% of all trees at high diversity levels (4- to 24-species mixtures) and 25% of all trees at low diversity levels (1 and 2 species) were examined every second or fourth row or column, as shown in Fig. 3. The direction of the survey was decided by the surveyor in the field in order to minimize the walking effort on slopes. In total, 27,249 individual trees of 26 species from 222 plots out of the 256 plots planted with trees were examined during the second survey.

2.8. Data analysis

Living seedlings were coded as “1” and dead ones as “0”. Tree positions that were not planted because of a shortage of seedlings or unsuitable site conditions (such as paths, rocks and cliffs) were not included. Ambiguous or unclear records were noted as missing values and also excluded from statistical analysis. The record of exact tree positions allowed us to assign to each observation the independent variables aspect, slope, curvature and elevation, as obtained from a digital elevation model (DEM). We used a 5 m DEM calculated by ordinary kriging with a nested variogram (Webster and Oliver 2001) based on a field campaign dataset (own differential GPS measurements). The overall quality of the DEM was high with an explained variance of 98 % and a root mean square error (RMSE) of 1.9 m (10-fold cross validation) in an elevation range of 112 m. All topographical calculations were done with ArcGIS 9.0 (ESRI Corp., Redlands, California, USA).

Seedling survival data of each of the two monitoring campaigns were analysed with generalized linear mixed effect models (GLMM), using a logit-link function and binomial error distribution (McCullagh and Nelder 1989). The fixed categorical factors were species richness level (1, 2, 4, 8, 16, 24 species) and leaf habit (deciduous, evergreen), fixed continuous factors were curvature, slope, altitude and Julian day of planting date in 2009. Species compositions nested in richness levels and plots nested in species compositions were included as nested random factors and species nested in leaf habit were included as a further crossed random factor in this model. As we did not aim at distinguishing the different scenarios, we neither considered scenario or the grouping of 4 mu-plots in this analysis. In a first step, linear mixed effects models were fitted that included all categorical and continuous factors and all their two-way interactions. In a second step, each model was optimised by removing insignificant interactions. Optimization was based on maximum subject-specific pseudo-likelihood (MSPL) parameter estimation and continued until the lowest –2 Residual Log Pseudo-Likelihood value was reached or when only significant effects and significant interactions remained in the model (Zuur et al. 2009). The probabilities and estimates of the final models were then calculated using residual subject-specific pseudo-likelihood (RSPL) estimation. We rerun the final models with the 177 and 175 plots of the first and second survey, respectively, which belonged to the total of 198 plots of the random partitions design, thus excluding the plots of the non-random extinction series and of the commercial species. To compare the impact of species identity on survival, a second model was run that retained all significant factors from the optimized first model but additionally included species identity as a fixed (rather than a random) factor. As a consequence of this moving of species identity from the random to the fixed effects terms, the contrast among species with different leaf habit had to be excluded from this model. This model only contained species compositions nested in richness levels and plots nested in species compositions as random factors. All statistical analyses were computed in SAS 9.2 (proc glimmix, SAS Institute Inc. 2006). Significance levels were based on type III sum of squares. Levels of fixed factors were compared using the Tukey-Kramer post-hoc test in the “lsmeans” statement. Graphs were produced from the models that used all monitored plots, thus also included the non-random extinction scenarios and monocultures of commercial species, using the least-square estimates and standard errors from the “lsmeans” and “estimate” statements in proc glimmix.

3. Results

Across all plots monitored, the mean survival rate across all species and plots was 57% in November 2009. It increased after the two replantings in November 2009 and March 2010 to 87% during the census interval April 2008–June 2010. The most important factors explaining the survival rate in November 2009 were diversity level, leaf habit, the interaction between diversity level and leaf habit, planting date (Julian day), and the interaction between planting date and leaf habit, whereas the survival rate in June 2010 was best explained by diversity level, leaf habit, diversity level x leaf habit and altitude (Table 2a and b). The variances explained by the random factors in the two models for the two monitoring dates differed in their relative contribution to the overall variance in survival. While in 2009 the variances of species compositions nested in richness, plots nested in species compositions and of species nested in leaf habit were 0.11 ± 0.06 (standard error), 0.59 ± 0.08 and 1.30 ± 0.39, respectively, the components were more similar in 2010 with 0.77 ± 0.21, 0.63 ± 0.10 and 1.21 ± 0.36, respectively. At both monitoring dates, most random variation was brought about by species identities.

For both survey dates, the diversity level of a plot had a significant impact on the survival rate, however, in different ways (Fig. 4a and b). At the end of the census interval April 2008–November 2009, there was a clear tendency of higher mortality and thus lower seedling establishment success at higher diversity levels. This continuous trend was no longer observed in the June 2010 survey after replanting. Until then, the highest mortality had occurred at the richness level 4, which was significantly different from richness level 8 according to the Tukey-Kramer post-hoc test. We tested whether this effect might have brought about by an over-representation of poorly performing species in plots of this richness level, by running the model only for the plots of the random partitions design in which every species was equally represented at every level of species richness. While the model for survival in November 2009 provided essentially the same result with decreasing survival rates with increasing richness levels (Table 2c), the richness effect disappeared for the second monitoring date (Table 2d).

During both census intervals, deciduous species had significantly higher survival rates than evergreen species (Fig. 5a and b). There was a significant interaction on seedling establishment between diversity level and leaf habit (Table 2): in 2009, the decrease in survival rates with increasing diversity was more pronounced for evergreen than for deciduous species (Fig. 4a), and in 2010, the reduction of survival in the 4-species mixtures was mainly affecting evergreen species (Fig. 4b).

The exact planting date (Julian day) had a negative effect on survival rate in 2009 (Table 2a). The later the seedlings were planted, the lower was their survival rate (Fig. 6). In addition, planting date interacted with leaf habit (Table 2a), indicating that later planting had a stronger negative impact on deciduous than on evergreen species (Fig. 6).

Among all the topographic factors examined, elevation was the only one with a significant positive effect on seedling survival in June 2010 (Table 2, Fig. 7). In contrast, aspect, slope inclination and curvature of the slope were not retained in the final models for both census intervals.

Not considering the leaf habit of species, survival rates in 2009 strongly varied among species, with exceptionally poor establishment in some evergreen species such as *Castanopsis carlesii*, *Castanopsis eyrei* and *Daphniphyllum oldhamii* (Fig. 8a). In contrast, the deciduous species *Choerospondias axillaris*, *Sapindus mukorossi* and *Melia azedarach* were most successful. Less variation in survival was observed in 2010, when after replanting most of the species could be established successfully with a survival rate >80% (Fig. 8b), except for five species, i.e. *Castanopsis carlesii*, *Castanopsis eyrei*, *Sapium discolour*, *Cyclobalanopsis myrsinifolia* and *Daphniphyllum oldhamii*.

4. Discussion

4.1. Determinants of seedling establishment

The overall establishment success after two planting campaigns clearly demonstrates the feasibility to establish a forest BEF experiment with a highly diverse species pool such as encountered in subtropical China. A survival rate after replanting of 87% exceeds the figures reported from other reforestation projects (e.g. Reubens et al. 2009). Although local forestry experience and other experimental attempts (such as Tsakaldimi et al. 2007; Villar-Salvador et al. 2004; Vilagrosa et al. 2003) suggest that evergreen broadleaved species are much more difficult to establish, they performed reasonably well in the plantation of the BEF China experiment. Still, evergreen species showed significantly lower establishment rates as compared to deciduous ones. The poor establishment of *Castanopsis carlesii*, *Castanopsis eyrei* and *Cyclobalanopsis myrsinifolia* was probably caused by poor quality of seedlings in 2009.

Given the large variation in aspect, slope and curvature at the planting site, we were surprised not to find any of these topographical variables to have a significant influence on survival, especially because even a much smaller variation in such variables had significant effects on tree growth (though not survival) in the Sardinilla BEF experiment in Panama (Potvin and Gotelli 2008; Healy et al. 2008; Scherer-Lorenzen et al. 2005a). However, also in plots near our BEF-China experiment we found that tree growth and morphology (in contrast to survival) was affected by slope (Lang et al. 2010). The only significant environmental explanatory factor that remained in the final model for the second census interval was elevation. The positive effect of elevation may have been due to winter temperature, because plots at higher elevation were less affected by cold air commonly accumulating in the valley bottoms. The importance of other abiotic or biotic site factors not measured directly can be deduced from the variance component of the random factor "plot" in the mixed-effects models, which contributed only 29.5% and 24.1% to the whole random variation for the first and second survey, respectively. The variance component of species composition increased seven-fold from the first to the second census period, demonstrating an increasing influence of the specific mixture of tree species in the plots. In contrast, the variance among species (within leaf habit) decreased, which suggests that species identity effects become less important when a plantation grows up.

That tree species richness had a significant effect on seedling survival at the first census is different from other BEF experiments where it had no effect (Potvin and Gotelli 2008; Healy et al. 2008). The most plausible explanation we can offer for this finding is that the planting of mixtures was more challenging than that of monocultures. It might well be that individual seedlings were not handled as carefully in more diverse than in less diverse plots. This effect remained even when accounting for planting date, as the diverse mixtures might also have been planted later, after the worker had gathered more experience with planting the monocultures. At the second monitoring date the 8-species mixtures had a signficantly higher survival rate (93±1.5%) compared to the 4-species mixtures (80±3.6%). We demonstrated that this effect was spurious by analysing only the plots of the random partitions design, where every species was represented the same number of times at every richness level. This showed that including the 48 plots of the non-random extinction scenarios resulted in a bias by including species with low establishment success more often at the tree richness level 4. This finding clearly shows the importance of a balanced design when evaluating richness effects in BEF experiments.

4.2. Practical issues for establishing polycultures in subtropical China

In our opinion, the establishment success of the BEF-China experiment was mainly brought about by sivicultural knowledge and careful planning. Of all practical issues briefly listed in Table 3, we consider planting date to be of paramount importance. In subtropical China, the most suitable planting time is from November to March and it differs between deciduous and evergreen species. Deciduous seedlings should be planted before bud break, while evergreen ones should be planted after winter when there is little frost risk, i.e. February and March. We had to cope with a delay in plot preparation in 2009, and started planting in late March. Daily maximum temperatures that continuously transgressed 25°C after 8 April have probably contributed to the high seedling mortality in plots planted later than that date. This issue was taken into consideration for replanting in 2010, when deciduous species were planted in November and evergreen ones in March.

The most important impediment to the wide-scale adoption of mixed- or multi-species plantations is the additional investment into the knowledge base that underpins the domestication, cultivation and use of each species (Bauhus and Schmerbeck 2010). In addition, operational-scale demonstration coupled with reliable financial analyses are needed to facilitate uptake of promising mixed-species models (Nichols et al. 2006; Knoke et al. 2008). For this purpose, we summarized some of the costs associated with the establishment of these mixtures in terms of labour days and money and compare them with conventional planting (Table 4). However, it should be noted that in our experimental plantations, species number was very high, seedlings were not available from commercial nurseries and the experimental objective required exact assignment of species to predefined planting positions. More relaxed requirements, such as planting small mono-specific clusters, would certainly reduce costs. We incurred about three times the costs of conventional planting. The most important cost factors in our experiment were manual site clearing and weeding. Conventionally, this is done through slash burning, which requires less than one worker per mu. In the BEF-China experiment, fire was excluded because carbon release by soil respiration and decomposition of remaining root systems and branches was studied in one of the BEF-China's subprojects. In addition, slash burning in short-rotation management of Chinese fir plantations have been identified as a major factor contributing to the yield decline observed in many places (Bi et al. 2007). Thus, the cost savings associated with slash burning may actually result in less earnings in the future. If the basic objective is to establish multi-species plantations, the main increases in expenditure are related to planting and weeding. This results in costs, which are less than two times those of conventional planting. At this point in time, considering previous experiences form BEF experiments carried out mainly in grassland (Quijas et al. 2010), we can only hypothesize that higher values of timber and ecosystem services will be obtained from our more diverse than on our less diverse plots and that this will more than offset the additional costs incurred at establishment (Bauhus et al. 2010).

5. Conclusions

We have shown that careful planning and a sufficient knowledge of siviculture and local phenology, plantations of evergreen species can be established in subtropical areas. This project has also demonstrated the feasibility of implementing mixed forest stands in the subtropics, even with species that previously have never been cultivated in plantations. The knowledge generated in this experiment can contribute to facilitate the use of mixed species plantations in the sub-tropics of China and elsewhere.

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Table 1: Species planted at site A of the BEF-China experiment in Jiangxi Province in 2009. Leaf habit: D = deciduous, E = evergreen. Successional stage as assessed from expert knowledge and from observations in the nearby Gutianshan National Nature Reserve (Yu et al. 2001; Bruelheide et al. 2011): E = early, I = intermediate, L = late. No. planted = number of seedlings that were planted across all 271 plots (see Fig. 1).

Species	Leaf habit	Successional stage	No. planted	Species	Leaf habit	Successional stage	No. planted
Acer davidii Franchet	D	E/I	1300	*Liquidambar formosana* Hance	D	I	4650
Castanea henryi (Skan) Rehder & E. H. Wilson	D	E	4650	*Lithocarpus glaber* (Thunberg) Nakai	E	I/L	7200
Castanopsis carlesii (Hemsley) Hayata	E	L	2100	*Melia azedarach* Linnaeus	D	E	1150
Castanopsis eyrei (Champion ex Bentham) Tutcher	E	L	5700	*Nyssa sinensis* Oliver	D	E	4450
Castanopsis sclerophylla (Lindley & Paxton) Schottky	E	E/I/L	6100	*Pinus massoniana* Lambert	E	E	2000
Choerospondias axillaris (Roxburgh) B. L. Burtt & A. W. Hill	D	E	4750	*Quercus acutissima* Carruthers	D	E	1550
Cinnamomum camphora (Linnaeus) J. Presl in Berchtold & J. Presl	E	E/I/L	1700	*Quercus fabri* Hance	D	E	4550
Cunninghamia lanceolata (Lambert) Hooker	E	E	2000	*Quercus serrata* Murray	D	E	5150
Cyclobalanopsis glauca	E	I/L	6950	*Rhus chinensis* Miller	D	E	4400

(Thunberg) Oersted							
Cyclobalanopsis myrsinifolia (Blume) Oersted	E	I/L	6000	*Sapindus mukorossi* Gaertn	D	E	4350
Daphniphyllum oldhamii (Hemsley) K. Rosenthal	E	L	1700	*Sapium discolor* (Champ.ex Benth.) Muell.-Arg	D	E	1350
Diospyros japonica Siebold & Zuccarini	D	E	1500	*Sapium sebiferum* (Linn.) Roxb	D	E	4300
Koelreuteria bipinnata Franchet	D	E	4250	*Schima superba* Gardner & Champion	E	E/I/L	6600

Table 2: Generalized linear effects model relating the survival rates of all planted tree species to diversity levels (1, 2, 4, 8, 16, 24 species), leaf habit (deciduous, evergreen) as categorical fixed factors, curvature (negative and positive values correspond to concave and convex slopes, respectively), slope, altitude and Julian day of planting date in 2009 as continuous fixed variables, as well as all twofold interactions. Plot nested within diversity level and species nested within leaf habit were included as random factors. **a)** and **c)** Optimized model for survival rates in November 2009, **b)** and **d)** optimized model for survival rates in June 2010. While models **a)** and **b)** used all plots monitored, models **c)** and **d)** used only the plots of the random partitions design where the number of occurrences of every species was fully balanced. All models are based on a binary-link function and binomial error distribution and on RSPL (residual pseudo-likelihood) parameter estimation. The tests of fixed effects are based on type III sum of squares, which makes them independent from the sequence they enter the model. Num df and Den df show degrees of freedom of numerator and denominator, respectively. P values for significant ($p < 0.05$) variables are shown in bold fonts.

Source of variation	Num df	Den df	F	P
a) Survival rate Nov. 2009 (all plots)				
Diversity	5	80	3.80	**0.0039**
Leaf habit	1	22	16.45	**0.0005**
Diversity x Leaf habit	5	30542	3.22	**0.0066**
Julian day	1	30542	15.17	**<0.0001**
Julian day x Leaf habit	1	30542	9.14	**0.0025**
b) Survival rate June 2010 (all plots)				
Diversity	5	80	2.56	**0.0333**
Leaf habit	1	22	4.99	**0.0360**
Diversity x Leaf habit	5	26999	2.85	**0.0142**
Altitude	1	26999	6.22	**0.0126**
c) Survival rate Nov. 2009 (random partitions design)				
Diversity	5	55	2.46	**0.0442**
Leaf habit	1	22	20.42	**0.0002**
Diversity x Leaf habit	5	23316	2.80	**0.0156**
Julian day	1	23316	5.28	**0.0215**
Julian day x Leaf habit	1	23316	10.74	**0.0011**
d) Survival rate June 2010 (random partitions design)				
Diversity	5	55	1.70	**0.1499**
Leaf habit	1	22	4.11	**0.0548**
Diversity x Leaf habit	5	21075	3.38	**0.0047**
Altitude	1	21075	7.32	**0.0068**

Table 3: Technical and practical issues in establishing polycultures.

	Technical and practical importance	Benefits and/or ecological concerns
Seeds harvesting	Obtain data on fruiting phenology of tree species	Adapt time for seed harvest, allow for repeated sampling over several months
	Check seed quality for germination capacity	Ensure sufficient seed quantity
	Store seeds in sand and cool environment	Ensure sufficient seed quality
Nursery practice	Provide good watering system	Ensure germination and seedling survival
	Provide shading and frost prevention facilities	Ensure seedling survival and quality
	Use decomposable containers	Time saving and lower damage risk for seedlings when planting
Seedling transportation and site storage	Transport the seedling in the coolest time of the day	Maintain high water potential of seedlings
	Trim 1/2 to 2/3 of number of branches before planting	Reduce water loss by respiration and decrease drought risk
	Water those seedlings in the morning and in the evening that are not directly planted and kept in temporary camps	Decrease drought risk
	Dip bare roots in muddy soil with added fertilizer (KH_2PO_4)	Protect roots from water loss and stimulate root growth
Planting	Involve experienced workers	Allows to adjust the procedure to unforeseen circumstances
	Set standard for the size and depth of the planting hole	Ensure the quality of planting practice
	Compress soil and slightly raise the plant after planting	Contributes to stretching the root system
Weeding	Pay attention to small seedlings not to erroneously remove them	Prevent seedling loss
	Lay the removed biomass around the seedling	Function as mulch
Seedling identity	Systematically tag the seedlings with long lasting material and water proof markers	Avoid accidental swapping of similar species
Planting position	Place bamboo sticks with species name marked at the devised planting positions	Time efficient when planting four species or more on predefined positions

Table 4: Cost comparison between conventional monoculture planting and establishing polycultures in the BEF-China experiment

Operation	Conventional planting	BEF-China experiment
Site preparation	<1 workers/mu for slash burning	7 workers/mu for cutting standing tree and removing above ground biomass
Planting preparation	Not required	1 worker/mu for placing bamboo sticks as markers for planting positions
Planting	1.5 workers/mu	2.5 workers/mu
Weeding (twice a year)	3 workers/mu	4-7 workers/mu
Seeds harvesting	Not required	200 worker day/year
Seedling	0.3 yuan/seedling (bare root);0.7 yuan/seedling (container)	0.3 yuan/seedling (bare root); 0.7 yuan/seedling (container)

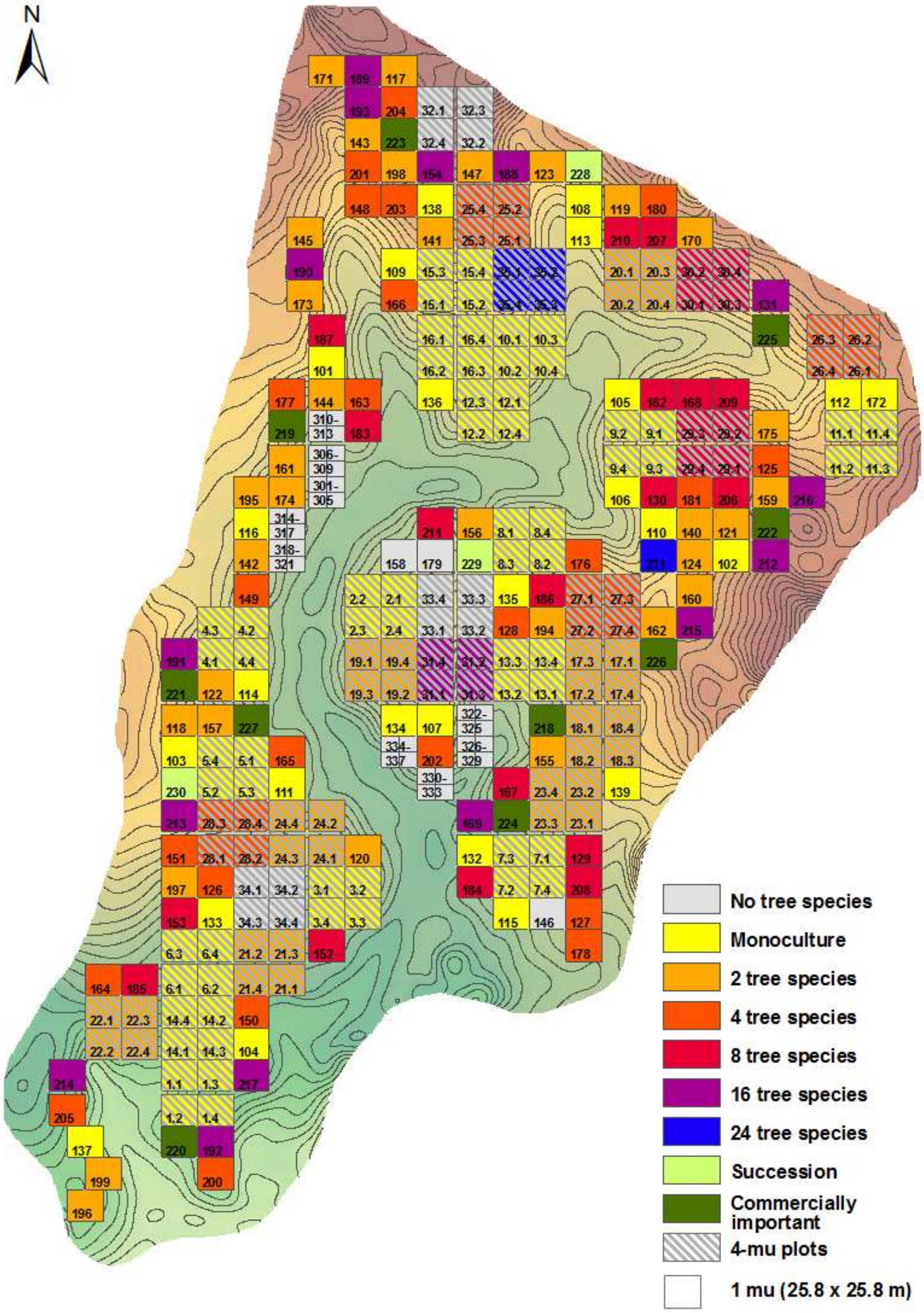


Fig. 1. Plot layout of site A of the BEF-China experiment, showing the 271 plots and the different treatments applied. Colours from yellow to blue show the number of tree species planted in a plot. Plots shown in grey color are treatments without trees. Plots in light green color are unmanaged plots, left to free succession, while plots in dark green are planted with commercially important trees, i.e. *Cunninghamia lanceolata* and *Pinus massoniana.*

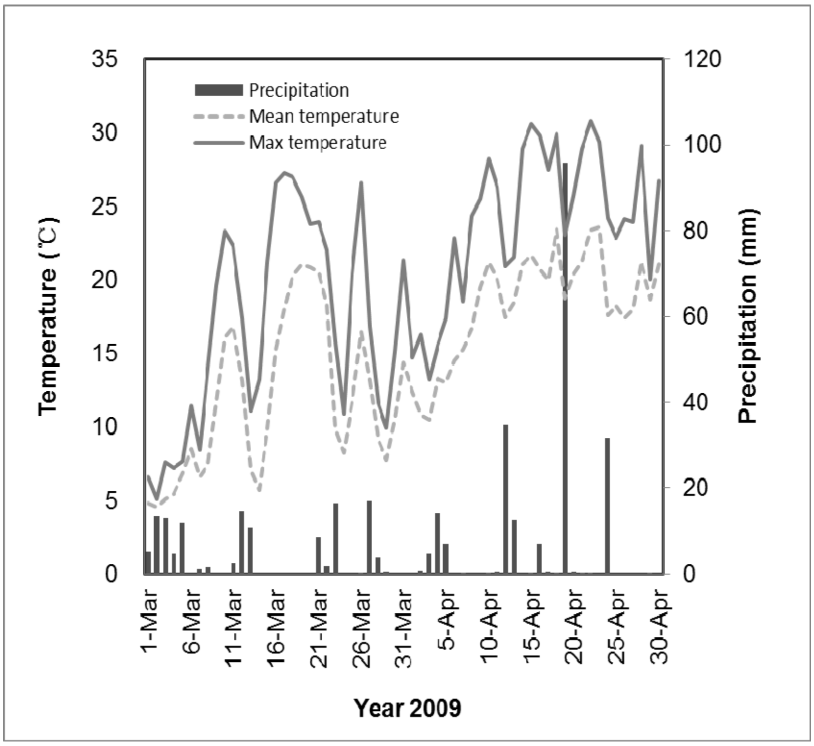


Fig. 2: Temperature and precipitation during the planting period in 2009

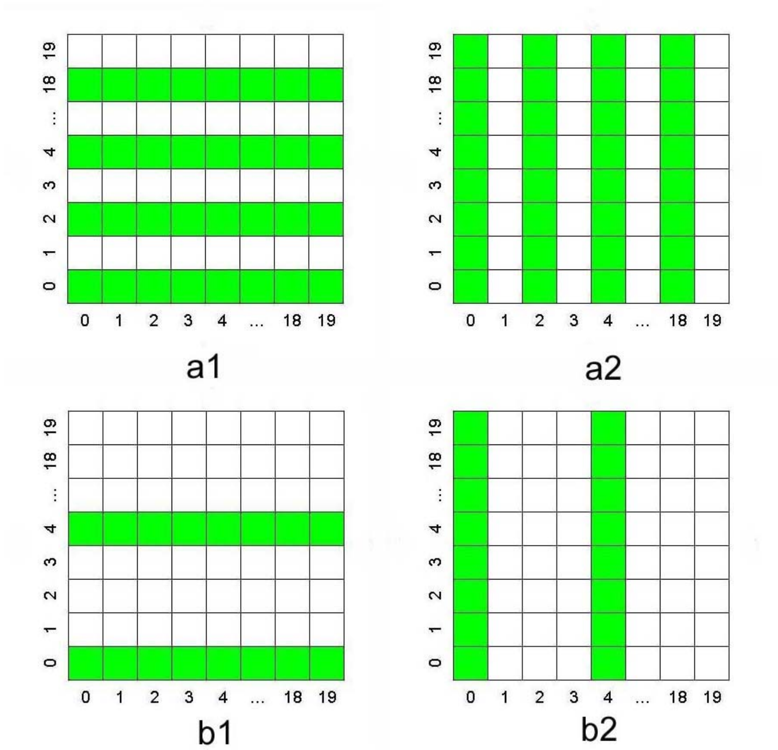


Fig. 3: Layout of the survey scheme in the June 2010 census. a1 and a2: at high diversity levels (4 to 24 species) every second row or column was surveyed; b1 and b2: at low diversity levels (1 and 2 species) every fourth row or column was surveyed. In total, there were 20 rows and columns (i.e. 400 trees) per plot.

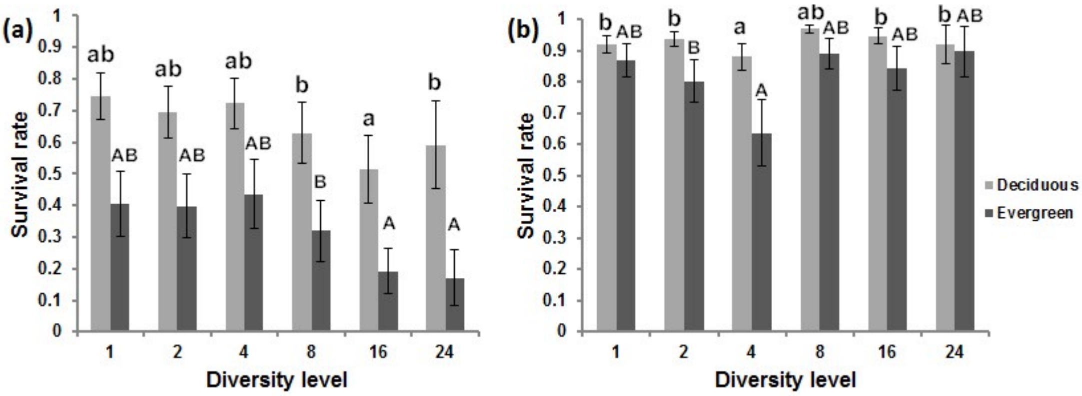


Fig. 4:Effect of tree richness level and leaf habit on tree sapling survival rates, using all plots monitored. (a) November 2009; (b) June 2010. Values are least square estimates and standard errors from the full model of Table 2a and b. Letters above bars indicate significant differences between values.

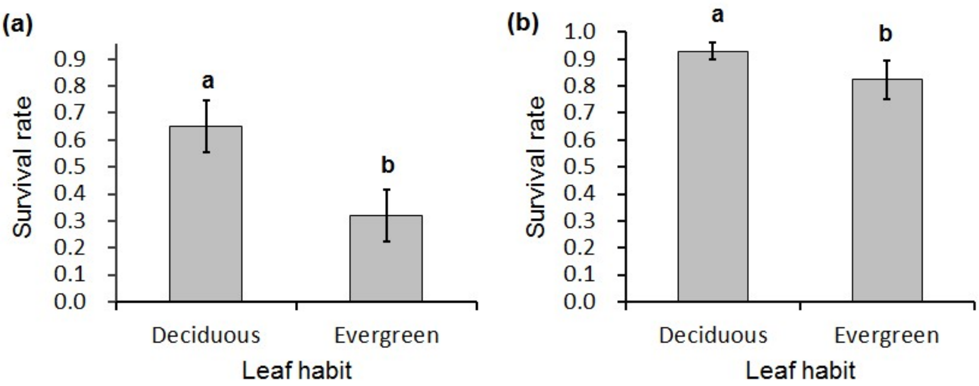


Fig. 5: Leaf habit effect on survival rate, using all plots monitored. (a) November 2009; (b) June2010. Values are least square estimates and standard errors from the full model of Table 2a and b. Letters above bars indicate significant differences between values.

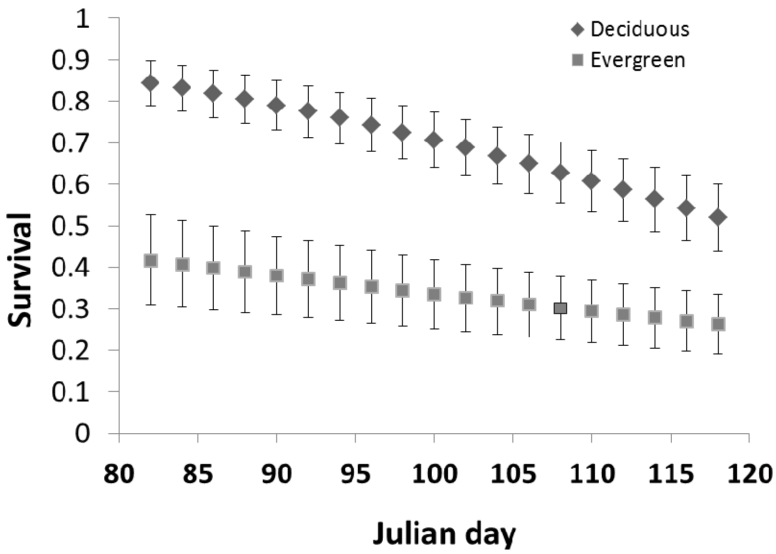


Fig. 6: Julian day x leaf habit effect on the survival rate surveyed in November 2009, using all plots monitored. Values are least square estimates and standard errors from the full model of Table 2a.

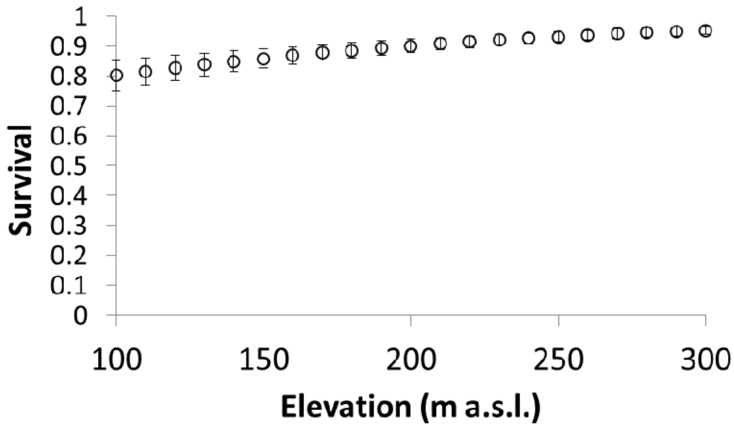
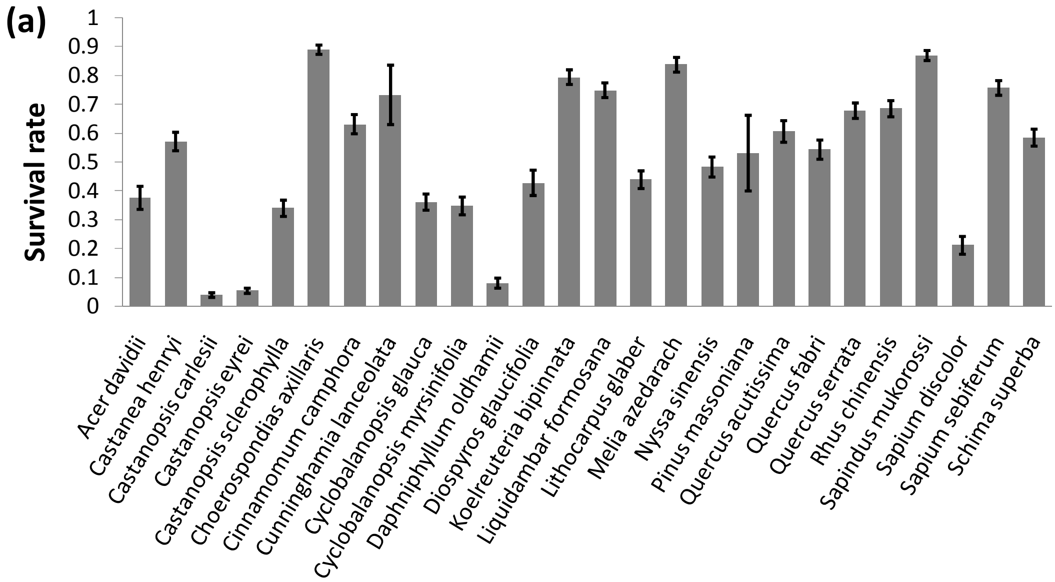


Fig. 7: Elevation effect on the survival rate surveyed in June 2010, using all plots monitored. Values are least square estimates and standard errors from the full model of Table 2b.



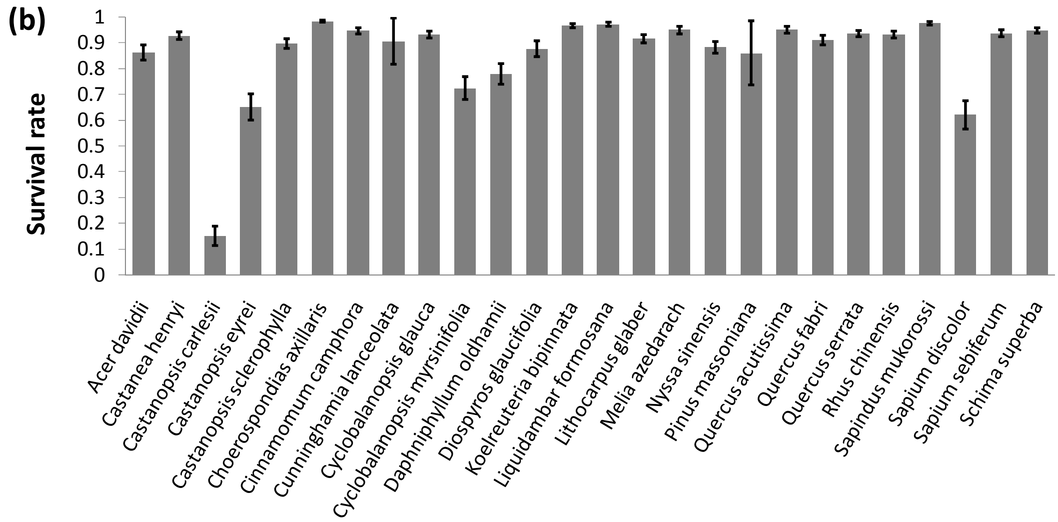


Fig. 8: Survival rates of the different species, (a) November 2009; (b) June 2010, using all plots monitored. Values are least square estimates and standard errors from a model similar to those in Table 2a and b, but including species identity as fixed factor and excluding leaf habit (see Methods).